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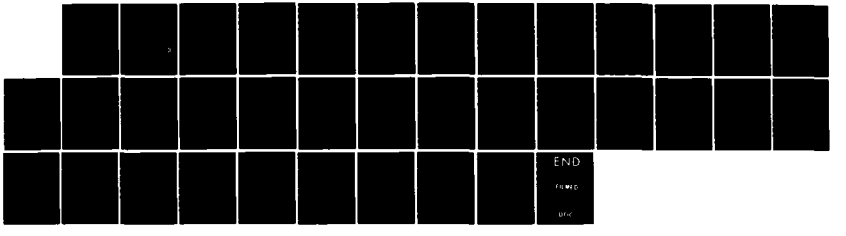

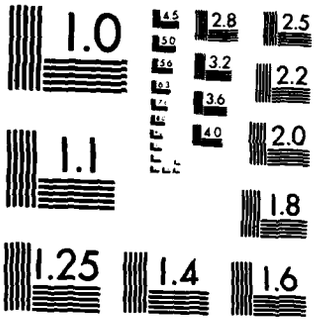

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# Evaluation of the Pharmacokinetics and Tolerance of Allopurinol Riboside in Human Volunteers

FINAL SCIENTIFIC REPORT

Theresa A. Shapiro, M.D., Ph.D.
Paul S. Lietman, M.D., Ph.D.

August 6, 1984

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD 17-83-C-3168

Division of Clinical Pharmacology
The Johns Hopkins University
School of Medicine
Baltimore, Maryland 21205



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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Allopurinol riboside is an experimental drug, of potential use in the treatment of leishmaniasis and Chagas' disease. A Phase I study of multiple doses of allopurinol riboside, to evaluate safety and pharmacokinetics, was conducted in 18 healthy male volunteers. Seven dosage levels were tested: 250, 500, 750, 1000, 1250, 1500 and 1750 mg, given on a qid schedule (1000-7000 mg/day) for a total of 13 doses. Two volunteers were evaluated at each level. Four additional volunteers received 1500 mg qid for 25 doses, over a period of 7 days. The study was conducted in three phases: screening, a five- or eight-day

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20 in-hospital phase, and a two-week follow-up phase. Safety monitoring included serial clinical evaluations, blood tests, electrocardiograms and urinalyses. Plasma and urine samples were collected for drug and metabolite determinations by high pressure liquid chromatography.

Allopurinol riboside was safe and well-tolerated by the volunteers in this study, in doses up to 7 grams/day for three days, or 6 grams/day for six days. Steady state plasma levels of allopurinol riboside with troughs exceeding 2 µg/ml are achievable. The metabolites, oxipurinol and allopurinol, appear in plasma and urine, in a dose-dependent fashion, but at the doses studied did not give rise to crystalluria or other signs of toxicity.

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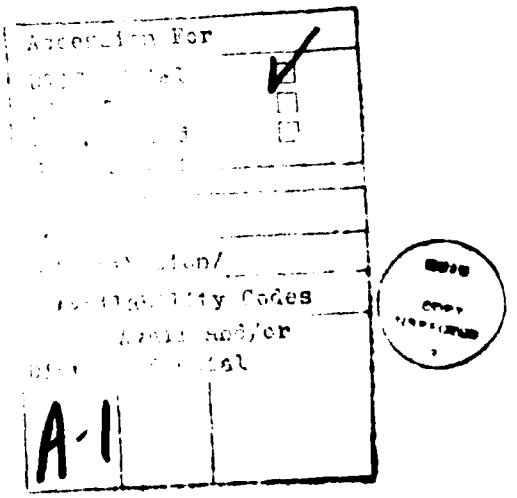

## FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45 CFR 46.

TABLE OF CONTENTS

## 1. INTRODUCTION

Allopurinol riboside (BW 28U) is a potential new drug for the treatment of cutaneous leishmaniasis, visceral leishmaniasis and Chagas' disease in man (1,9,11). The present accepted therapy of leishmaniasis with pentavalent antimonials, while widely employed and frequently effective, is rendered extremely difficult and impractical by the need for repeated parenteral dosing with substantial daily injections, often for very extended periods of time. Nifurtimox and benzonidizole, the drugs currently available for treatment of Chagas' disease, cause dermatologic reactions in up to 50% of recipients, and a potentially debilitating peripheral neuropathy has been observed in 30 to 40% of cases. Other less serious but frequent reactions include generalized weakness, drowsiness, anorexia, nausea and vomiting.

Clearly a safe, orally-effective drug for the treatment of leishmaniasis and Chagas' disease is needed. This goal has, in fact, been accorded recognition and a high degree of priority among the six targeted disease categories of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases.

The selection of allopurinol riboside as a potential new drug for the treatment of leishmaniasis and Chagas' disease is based on studies of the enzymology and biochemical pathways of purine and nucleic acid metabolism in these parasites and in mammalian cells. This work took place on a background of extensive knowledge of the clinical effects and mode of action of allopurinol (3,5,6,8,10).

Since the parasite is able to utilize allopurinol as a starting substrate to form allopurinol ribotide, it would seem to be an attractive candidate as a potential antileishmanial and antitrypanosomal drug. However, allopurinol in man is rapidly metabolized to oxipurinol; only a small proportion is converted to the riboside or the ribotide (4). Oxipurinol and its riboside are relatively inactive against the parasite. On the other hand, allopurinol riboside is not split back to the base and is not directly phosphorylated in mammalian cells (7). It is largely available, therefore, as allopurinol riboside to the parasite for phosphorylation, amination and incorporation into its nucleic acids.

The authors have conducted a Phase IA study of the safety, tolerance and pharmacokinetics of allopurinol riboside in 15 normal male volunteers. Doses of 0 (placebo), 5, 10, 15, 20 and 25 mg/kg were administered to two individuals at each level except for the last, 25 mg/kg, which was administered to 5 volunteers, four of whom completed the protocol. The participants were monitored for objective and subjective side effects. Periodic laboratory evaluations included measurements of hematocrit, total and differential white blood cell count, platelet count, prothrombin time and serum electrolytes, BUN, creatinine, GOT, GPT, total bilirubin, alkaline phosphatase and uric acid. Urinalyses (chemical and microscopic) were also performed. Plasma and urine specimens were obtained for detailed pharmacokinetic analysis.

Minor symptoms were reported by three of the volunteers, who received 5 or 10 mg/kg allopurinol riboside. There were no significant changes in the physical examinationss (including vital signs), hematology or chemistry evaluations, or in the urinalyses of any of the volunteers, for up to two weeks after drug administration. We concluded that allopurinol riboside was safe and well tolerated in this study at single oral doses up to and including 25 mg/kg.

Preliminary pharmacokinetics data analyses from this study indicate that the absorption of allopurinol riboside was incomplete and probably not linear with respect to the dose. The terminal elimination half-life in the plasma at all doses was betweeen 3 and 4 hours. A surprising finding in this study was the appearance in plasma and urine specimens of oxipurinol, particularly at doses of 15, 20 and 25 mg/kg. Peak concentrations of allopurinol riboside were observed approximately 1 hour after dosing and ranged from 2.3 to 7.3 ug/ml at doses of 10 mg/kg or higher, except in one individual who had a peak concentration of 10.2 ug/ml following a dose of 25 mg/kg. Otherwise, the peak concentrations were not proportional to the dose above 10 mg/kg. Urinary recovery of allopurinol riboside 48 hours after dosing ranged from 15-48% of the administered dose. Oxipurinol did not appear in the plasma until hours after dosing and gradually reached peak concentrations at about 24 hours. The highest concentrations of oxipurinol were observed at the highest dose, and approached 6 ug/ml. The plasma half-life of oxipurinol is known from previous studies with allopurinol to be approximately 30 hours.

The appearance of oxipurinol as a metabolite in this study was unexpected. There is no known mechanism by which allopurinol riboside can be metabolized to oxipurinol in man (4). Indeed, part of the rationale for the development of allopurinol riboside rather than allopurinol as an antileishmanial/antitrypanosomal drug was to obtain adequate levels of the active drug without the accumulation of oxipurinol, which occurs with high doses of allopurinol. Oxipurinol blood concentrations as high as 20-30 ug/ml are observed in patients taking 900-1200 mg per day of allopurinol and are not associated with any particular risk. However, excretion of oxipurinol in the urine at levels in excess of 300-400 mg/L may constitute some risk due to the poor solubility of this compound.

## 2. MATERIALS AND METHODS

### 2.1 Allopurinol riboside and placebo

Allopurinol riboside was provided by the Burroughs-Wellcome Company as a white powder in colorless gelatin capsules, each containing 250 mg of drug. Placebo capsules, identical to those containing allopurinol riboside, were also provided by the Burroughs-Wellcome Company.

### 2.2 Subjects

Healthy men who were able to give written informed consent were eligible to volunteer for the study. The study was approved by the Joint Committee on Clinical Investigation of The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital.

#### 2.2.1 Inclusion Criteria

To participate in the study the volunteer had to be male, between 18 and 50 years old and within 10% of ideal body weight for his height. A detailed health history and physical examination were performed by a physician. Serum chemistries, hematology and urine analysis had to be within the normal ranges as defined by The Johns Hopkins Department of Laboratory Medicine, and electrocardiogram had to be normal.

#### 2.2.2 Exclusion Criteria

Women were excluded from this study. Medical students and others who might be considered coerced into volunteering were not eligible to participate. Men were excluded if they did not meet the criteria listed above (2.2.1), if they were chronic users of any drugs, or had a known or suspected allergy to allopurinol. Once accepted as candidates for the study, subjects were not permitted to take any drugs for one week prior to admission to the Clinical Research Unit.

#### 2.2.3 Recruitment

Advertisements were placed in the help wanted classified sections of metropolitian Baltimore newspapers. A special telephone line was dedicated to volunteer recruitment. Interested candidates were screened on the telephone by a research nurse who described the details of the study, took a brief history and scheduled the appropriate screening examinations.

2.2.4 Description of Population of Subjects

During the course of the study, a total of 5 newspaper advertisements were placed, and 141 men responded. From this group, 19 were eventually entered into the study. A list of the reasons for rejecting potential volunteers is as follows:

| | |
|---|---|
| 141 | Responded to newspaper ads |
| -41 | Rejected on telephone |
| 127 | Scheduled for screening |
| -59 | Failed to keep screening appointments |
| 68 | Seen at Johns Hopkins |
| -33 | Abnormal screening laboratory test |
| 35 | Examined by physician |
| -4 | Failed history or physical exam |
| 31 | Acceptable men |
| -10 | Changed mind, moved, etc. |
| 21 | Admitted to Johns Hopkins |
| -2 | Failed admission laboratory tests |
| 19 | Entered study |
| 18 | Completed study |

One volunteer (008) did not complete the study, because his liver function tests, which returned after he had received two doses of allopurinol riboside, were found to have been abnormal before he received any drug (see 3.1.2.1). Of the 18 who completed the study, 11 were caucasian and 7 were black. The average age was 32 years ranging from 20 to 48 years old.

2.2.5 Informed Consent

Written informed consent was obtained from each participant upon admission to the Clinical Research Unit. This document described in detail the purpose of the study, the protocols, and the potential risks.

2.2.6 Compensation

A payment schedule was designed to compensate volunteers for their participation.

| | |
|---|---|
| Screening phase | no compensation |
| In-hospital phase | $100.00 |
| Follow-up ambulatory phase | 50.00 |
| Bonus for completion of study | 20.00 |
| Total for entire study | $170.00 |

Subjects in the second part of the study, which require three additional in-hospital days, received a total of $300.

### 2.2.7 Liability

Liability for unexpected toxicity was provided by the U.S. Army, and for malpractice by The Johns Hopkins Medical Institutions.

## 2.3 Study Design

The study was a rising-dose clinical trial to evaluate the safety, tolerance, and pharmacokinetics of multiple doses of allopurinol riboside in human volunteers.

Seven dosage levels were tested: 250, 750, 1000, 1250, 1500, and 1750 mg. At each level, 13 doses of allopurinol riboside were administered on a q.i.d. schedule (7 a.m., 12 noon, 5 p.m. and 10 p.m.) over 4 days (total dose 3.25 - 22.75 gms). Two volunteers were evaluated at each level, and no volunteer was treated more than once. All drugs were administered by an investigator or research nurse employed by the study.

Provision was made to repeat the administration of a given dose of drug, should a question of side-effects arise. This side loop would have been conducted with placebo controls, in a randomized, doubled-blinded fashion.

Originally, the study was to include an additional four subjects at the highest tolerated dose. However, the unexpected appearance of significant levels of the metabolite oxipurinol led to a change in design: the four additional volunteers received 1500 mg of allopurinol riboside q.i.d. for 25 doses, over a period of 7 days.

The study was conducted in three phases. The screening phase, a five or eight day in-hospital phase, and a two week follow-up phase. The procedural timetable for safety monitoring, and plasma and urine collection for pharmacokinetics is shown in Appendix A.

## 2.4 Laboratory Examinations

All laboratory examinations were done within The Johns Hopkins Medical Institution. Hematology, chemistry, virology and serology determinations were performed by the Department of Laboratory Medicine (Clinical Laboratory License number 19-1054).

2.4.1 Hematology

Routine hematologic determinations, including hematocrit, hemoglobin, red blood cell count, white blood cell count with differential count, and platelet count were done on a coulter counter. Prothrombin time was measured by a Lancer Coagulyzer.

2.4.2 Chemistry

Serum was assayed for sodium, potassium, chloride, carbon dioxide, urea nitrogen, creatinine, glucose, total bilirubin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase.

2.4.3 Electrocardiography

Standard 12 lead EKG tracings with one minute rhythm strips were taken on admission to the hospital. Electrocardiograms were interpreted by a cardiologist on the staff of The Johns Hopkins Hospital.

2.4.4 Miscellaneous

Urine analyses were performed in the laboratories of the Division of Clinical Pharmacology. Protein, glucose, ketones and bilirubi were measured qualitatively, and pH was determined. A microscopic examination of the sediment was performed.

If a subject developed serum transaminase levels above the upper limits of normal, an additional series of laboratory tests was done to rule out possible disease states. This battery included hepatitis B surface antigen and antibody, antibody to hepatitis B core antigen, antibody to hepatitis A virus, and hepatitis B "e" antigen. In addition, a mononucleosis screen was performed on serum. Urine and blood (buffy coat) were cultured for cytomegalovirus (CMV) and acute and convalescent serum CMV titers were measured.

2.5 Drug and metabolite determinations

A method has been developed by Dr. Donald Nelson of the Wellcome Research Laboratories, and modified by the authors, for the perchloric acid extraction of allopurinol riboside and all of its known metabolites in plasma and urine samples. Analyses were performed by high pressure liquid chromatography, using an octadecylisilane reversed phase column. Quantitation was based upon UV peak areas obtained from the HPLC elution profile. Linear recoveries were obtained between 0.2 and 50 ug/ml and results were corrected for recovery of an internal standard,

N-acetyl-p-aminophenol, added to the extraction mixtures. From a single analysis, the following compounds were determined: uric acid, hypoxanthine, xanthine, inosine, allopurinol, oxipurinol, allopurinol riboside, oxipurinol riboside and tryptophan. In over 150 determinations, the standard deviation was less than 15% of the mean for allopurinol riboside, 10% for oxipurinol, and 8% for allopurinol.

### 2.6 Pharmacokinetic Calculations

The areas under the plasma concentration-time curves were calculated with the aid of a Wang System 2200 computer, programmed to utilize both log-linear and linear methods, with extrapolation to infinity. Both methods gave comparable results.

The terminal plasma half-lives were calculated using the relationship

$$\log C = -kt \log Co.$$

with eight time points from 4 to 12 hours after drug administration. The slope of the line (k) was obtained by least squares regression analysis on a Model TI55 Texas Instruments calculator, and substituted into

$$t\ 1/2 = \frac{0.693}{k}$$

to obtain the half-life.

## 3. RESULTS AND DISCUSSION

### 3.1 Clinical

#### 3.1.1 Symptomatic

Clinical complaints were minimal, from two subjects. One week after the final dose of allopurinol riboside, a subject who received 250 mg q.i.d. for 13 doses (001) developed a 3 cm pruritic papular rash behind his right knee, which resolved in two weeks. He attributed this to poison ivy. On the first day of treatment, another volunteer, who received 1000 mg q.i.d. (007), complained of a headache, which lasted two hours and required no treatment, and of three loose stools, which resolved by the next morning. No other volunteers reported any symptoms.

### 3.1.2 Laboratory

#### 3.1.2.1 Liver function tests

A subject who received 750 mg q.i.d. doses (006) developed marginally elevated serum transaminase levels, with a peak SGOT of 47 IU/L (day 6 of study, normal <41) and a peak SGPT of 46 IU/L (day 6 of study; normal <33). Alkaline phosphatase and total bilirubin were unaffected, and there were no clinical signs or symptoms of hepatitis. He admitted to alcohol consumption during the study. His enzyme levels returned to normal in two weeks, and remained normal one week thereafter. The following laboratory tests for infectious hepatitis were negative: hepatitis B surface antigen and antibody, hepatitis B core antibody, hepatitis A antibody, mononucleosis spot test, VDRL, cytomegalovirus titers, and urine and buffy coat cultures for cytomegalovirus.

One subject who received 750 mg q.i.d. X 13 doses (005) developed elevated serum transaminase levels two weeks after discharge from the hospital: SGOT 65 IU/L, and SGPT 79 IU/L. These values returned to normal six days later, and remained so for two weeks thereafter. He had no elevations of bilirubin or alkaline phosphatase, and no clinical symptoms or signs of hepatitis. He denied alcohol consumption. All laboratory tests for infectious hepatitis (see previous subject) were negative, except a positive VDRL and FTA, which were addressed by the public health service.

One subject (008) with a long record of normal SGPT values in this unit (including those taken at screening and on the day of admission), and in the Community Blood and Plasma Service in Baltimore, was discharged from the study after receiving two doses of 1000 mg allopurinol riboside, because his SGOT and SGPT levels were elevated (66 and 44 IU/L, respectively) on the morning of the first dose, before drug was given. He was followed for two weeks, during which his transaminase levels fell to normal limits.

#### 3.1.2.2 Uric Acid Levels

All subjects, except the two at the lowest dosage level, had a decrease in serum uric acid levels to less than 4.2 ml/dl. This finding is attributable to the inhibition of xanthine oxidase by the metabolite, oxipurinol.

### 3.1.3 Conclusions

Allopurinol riboside was safe, and well-tolerated by the volunteers in this study, in doses up to 7 grams/day for three days, or 6 grams/day for six days.

Three volunteers had mild, reversible elevations of serum transaminase levels. In 008, this occurred before drug was given; he was discharged from the hospital (with a two week outpatient follow-up) and replaced. In 006, the changes were not clinically significant (more than twice the upper limits of normal), as defined by the NIH "Guidelines for Detection of Hepatotoxicity due to Drugs and Chemicals" (1978, ref. 2), and they occurred in the context of alcohol consumption. In 005, only SGPT elevations were significant.

The possibility that allopurinol riboside causes transaminase elevations in 005 and 006 cannot be ruled out entirely. However, it seems unlikely for two reasons. First, the time course was different in these two men (onset day 6 for 006, and day 17 for 005), Second, and more importantly, ten additional volunteers received higher doses of allopurinol riboside, some for a longer time, without developing any evidence of hepatotoxicity.

The only other significant laboratory changes seen in this study were decreases in serum uric acid levels, expected from the presence of allopurinol and oxipurinol.

## 3.2 Pharmacokinetics and Metabolism

The kinetics and metabolism of allopurinol riboside were largely predictable from the results of the single dose studies.

### 3.2.1 Allopurinol riboside

As in the single dose study, the absorption of allopurinol riboside appears to have been incomplete. Neither peak plasma levels (Figs. 1 and 2) nor 24 hour urinary excretion (Fig. 3) of allopurinol riboside was dose dependent, although the sample number may be too small to conclude this with certainty.

Plasma levels of allopurinol riboside peaked between one and two hours after drug administration, and plasma elimination appears to have been from a single compartment (Fig. 1). There was no evidence for allopurinol riboside accumulation.

After 13 doses, the mean half-life of elimination of allopurinol riboside from plasma was 3.50 $\pm$ 1.09 hours (Table 1), which is not significantly different from that after a single dose (3.05 $\pm$ 0.78 hours; $p = 0.11$).

At steady state, after a dose of 1500 mg q.i.d., plasma levels of allopurinol riboside fluctuated between a mean minimum of 2.5 ug/ml and a mean maximum of 5.6 ug/ml (Table 2).

3.2.2 Metabolites

The appearance of oxipurinol and allopurinol in plasma and urine was an unexpected finding of the single dose study, which was corroborated in this multiple dose study. After a single dose of allopurinol riboside, up to 60% of the administered drug could be accounted for by urinary excretion of *allopurinol riboside* (17-50%), oxipurinol (0-20%) and allopurinol (0-16%). The urinary excretion of allopurinol riboside, oxipurinol and allopurinol during the 24 hours after the final dose of allopurinol riboside are shown in Figure 4. At steady state in subjects receiving 1500 mg q.i.d. allopurinol riboside, up to 41% of the dose was excreted in the urine, as allopurinol riboside (10-29%), oxipurinol (5-10%), and allopurinol (2%; Table 3).

Oxipurinol appears in plasma more than four, and in most cases, 15 hours after administration of the first dose of allopurinol riboside (Fig. 1). Steady state levels of oxipurinol are attained by 72 hours after the first dose of allopurinol riboside, and are dose-dependent (Fig. 5). No subject had plasma levels of oxipurinol greater than 27 ug/ml.

Total urinary excretion of oxipurinol over 24 hours after the final dose of allopurinol riboside was also dose-dependent (Fig. 6). The maximum 24 hour urinary excretion of oxipurinol was 647 mg, in a subject (013) who received 13 doses of 1750 mg (24.4 mg/kg) allopurinol riboside. The concentration of oxipurinol in his urine during this period ranged between 323 and 510 mg/L. In all other subjects, urine oxipurinol concentrations were less than 400 mg/L. No crystalluria was seen in any subjects.

Allopurinol was measurable in the plasma, 16 or more hours after the first dose of allopurinol riboside, in subjects who received doses of allopurinol riboside greater than 1250 mg. These levels tended to be dose-dependent (Fig. 7), and did not exceed 2.4 ug/ml (seen in volunteer 013).

3.2.3 Conclusions

With multiple oral doses of allopurinol riboside, given on a q.i.d. schedule (7 a.m., 12 noon, 5 p.m., 10 p.m.), steady state plasma levels with troughs exceeding 2 ug/ml are achievable. The metabolites oxipurinol and allopurinol appear in plasma and urine, in a dose-dependent fashion, but at doses up to 7 grams/day for three days, and 6 grams/day for 6 days do not give rise to crystalluria or other signs of toxicity.

On the basis of these findings, several recommendations for efficacy studies in man can be made. First, it would be reasonable to evaluate doses of about 20-25 mg/kg. In this range, the plasma concentrations of allopurinol riboside are maximum, and oxipurinol levels are accepted. Second, patients should be kept well-hydrated, to reduce the urinary concentrations of oxipurinol. Third, patients should be monitored, ideally for drug and metabolite concentrations in plasma and urine, but certainly for evidence of crystalluria.

Publications supported by the contract, and personnel receiving contract support are listsed in Appendices B and C.

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Table 1

Allopurinol Riboside: T 1/2 Disappearance from Plasma after Multiple Oral Doses

| Volunteer number | Allopurinol riboside doses (mg) | Allopurinol riboside doses (mg/kg) | Allopurinol riboside total number of doses | T 1/2 (hours) |
|---|---|---|---|---|
| 001 | 250 | 4.02 | 13 | 2.09 |
| 002 | 250 | 4.17 | 13 | 2.64 |
| 003 | 500 | 8.96 | 13 | 2.40 |
| 004 | 500 | 7.89 | 13 | 3.53 |
| 005 | 750 | 11.2 | 13 | 1.99 |
| 006 | 750 | 9.57 | 13 | 3.21 |
| 007 | 1000 | 12.7 | 13 | 2.43 |
| 108 | 1000 | 11.6 | 13 | 4.44 |
| 009 | 1250 | 20.8 | 13 | 3.65 |
| 010 | 1250 | 15.8 | 13 | 5.40 |
| 011 | 1500 | 22.5 | 13 | 4.60 |
| 012 | 1500 | 21.5 | 13 | 3.57 |
| 013 | 1750 | 24.4 | 13 | 4.63 |
| 014 | 1750 | 25.0 | 13 | 4.48 |
| 015 | 1500 | 20.4 | 25 | 4.31 |
| 016 | 1500 | 18.9 | 25 | 2.44 |
| 017 | 1500 | 23.5 | 25 | 4.92 |
| 018 | 1500 | 22.8 | 25 | 4.79 |

T 1/2 disappearance from plasma after the final dose of allopurinol riboside, calculated as described in 2.6. Mean $\pm$ standard deviation for T 1/2 is 3.50 $\pm$ 1.09.

Table 2
Allopurinol Riboside: Peak and Trough Plasma levels

| Volunteer number | Plasma levels (ug/ml) peak | trough |
|---|---|---|
| 011 | 9.82 | 2.72 |
| | | 2.69 |
| | | 3.19 |
| 012 | 4.79 | 1.83 |
| | | 2.70 |
| | | 3.29 |
| 015 | 6.25 | 2.36 |
| | 5.30 | 2.13 |
| | | 3.98 |
| | | 2.83 |
| | | 1.97 |
| | | 1.67 |
| 016 | 6.39 | 3.32 |
| | 10.04 | 4.29 |
| | | 3.65 |
| | | 1.92 |
| | | 3.23 |
| | | 4.22 |
| 017 | 2.78 | 1.66 |
| | 2.50 | 2.74 |
| | | 1.93 |
| | | 2.95 |
| | | 2.00 |
| | | 0.79 |
| 018 | 6.27 | 3.20 |
| | 2.06 | 1.58 |
| | | 1.94 |
| | | 2.22 |
| | | 1.09 |
| | | 1.76 |

All subects received 1500 mg allopurinol riboside q.i.d. Numbers 011 and 012 had a total of 13 doses, and the others had a total of 25 doses. Peak levels were measured after the final dose of allopurinol riboside, and in numbers 015-018, also after the 23rd dose. All trough levels were taken just before the 7 a.m. dose.

Table 3
Urinary Excretion of Allopurinol Riboside
and Metabolites at Steady State

| Vol. no | Allopurinol riboside | | Amount in Urine[a] (mg) | | | Total | |
|---|---|---|---|---|---|---|---|
| | mg/kg per dose | 24 hr total, mg | Allopurinol riboside | Oxipurinol | Allopurinol | mg[b] | %dose |
| 015 | 20.4 | 6000 | 1486 | 293 | 57 | 2115 | 35 |
| 016 | 18.9 | 6000 | 1761 | 354 | 47 | 2478 | 41 |
| 017 | 23.5 | 6000 | 571 | 155 | 63 | 968 | 16 |
| 018 | 22.8 | 6000 | 862 | 160 | 61 | 1264 | 21 |

[a]Total amount excreted during a 24 hour period; values are averages from days 4,5 and 6 of drug administration. [b]Total "allopurinol riboside equivalents" calculated on the basis of molecular weights: 152.11 (oxipurinol), 136.11 (allopurinol) and 268.24 (allopurinol riboside).

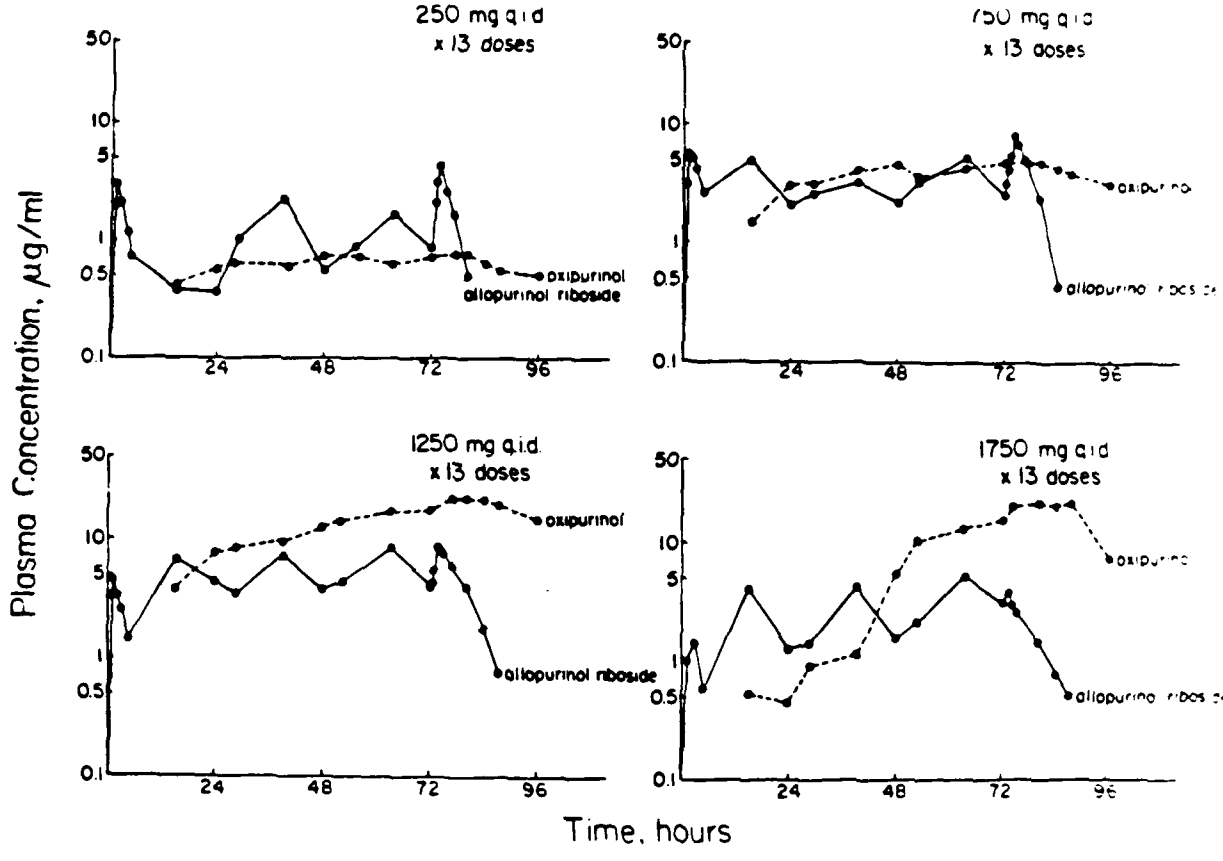


Figure 1. Representative plasma concentrations of allopurinol riboside and oxipurinol. Profiles from four representative volunteers (001, 006, 009, 013) at different dose levels are displayed. Unlike oxipurinol plasma levels, allopurinol riboside concentrations do not increase appreciably with dose.

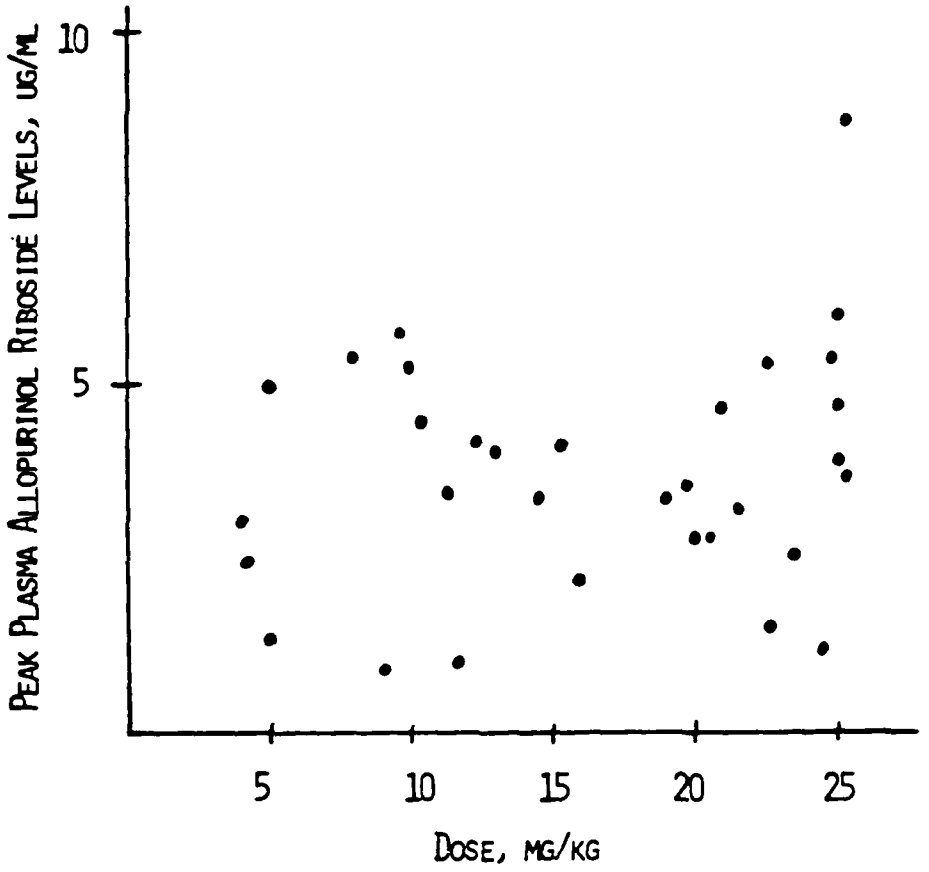


Figure 2. Peak plasma levels of allopurinol riboside versus dose. These data include peak concentrations from the single dose study, and from the first dose of the multiple dose study. There is no apparent dose-dependence.

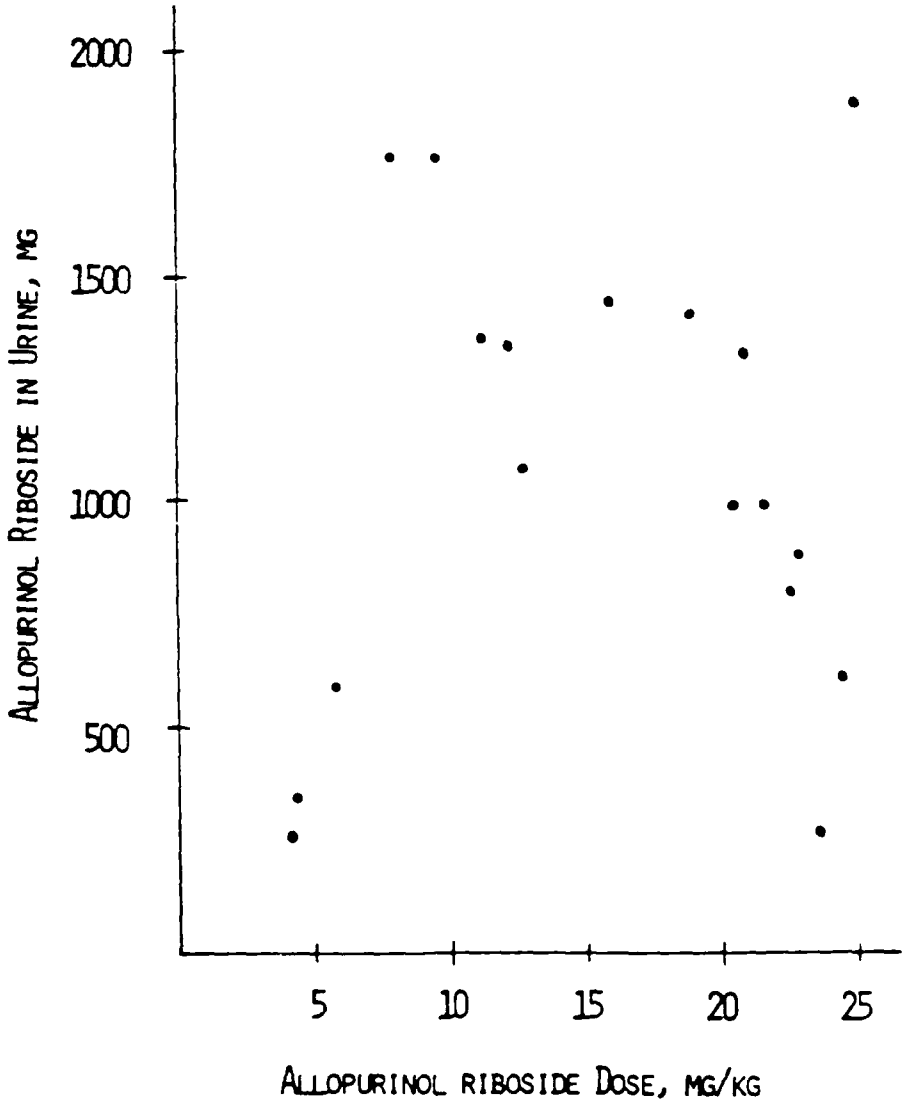


Figure 3. Urinary excretion of allopurinol riboside versus dose. Urine was collected for 24 hours after the initial dose of allopurinol riboside. No dose-dependence is evident.

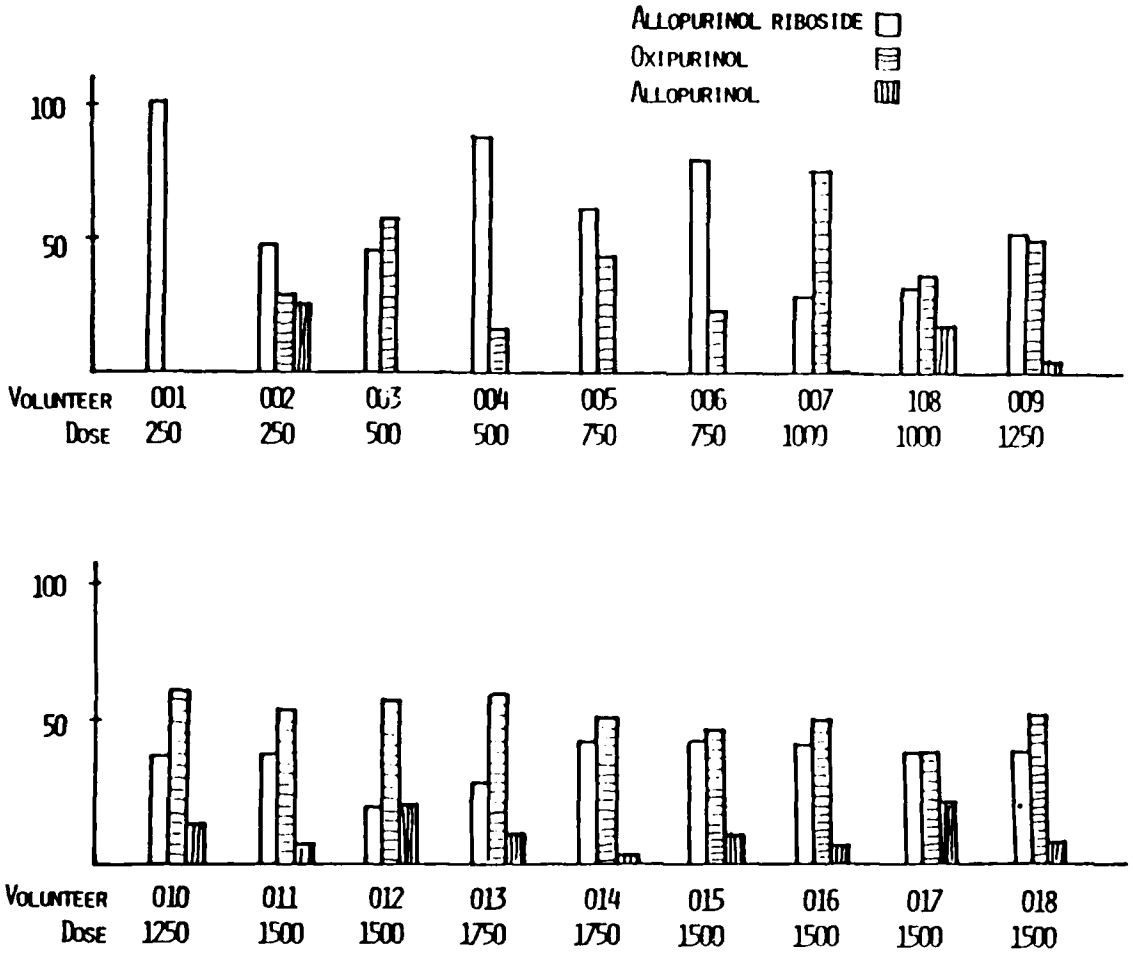


Figure 4. Pattern of urine metabolites after multiple doses of allopurinol riboside. Urine was collected for 24 hours after the final dose of allopurinol riboside. Oxipurinol and allopurinol were converted to "allopurinol riboside equivalents" on the basis of molecular weights. Allopurinol riboside and its metabolites are depicted as percentages of the total amount of "allopurinol riboside"

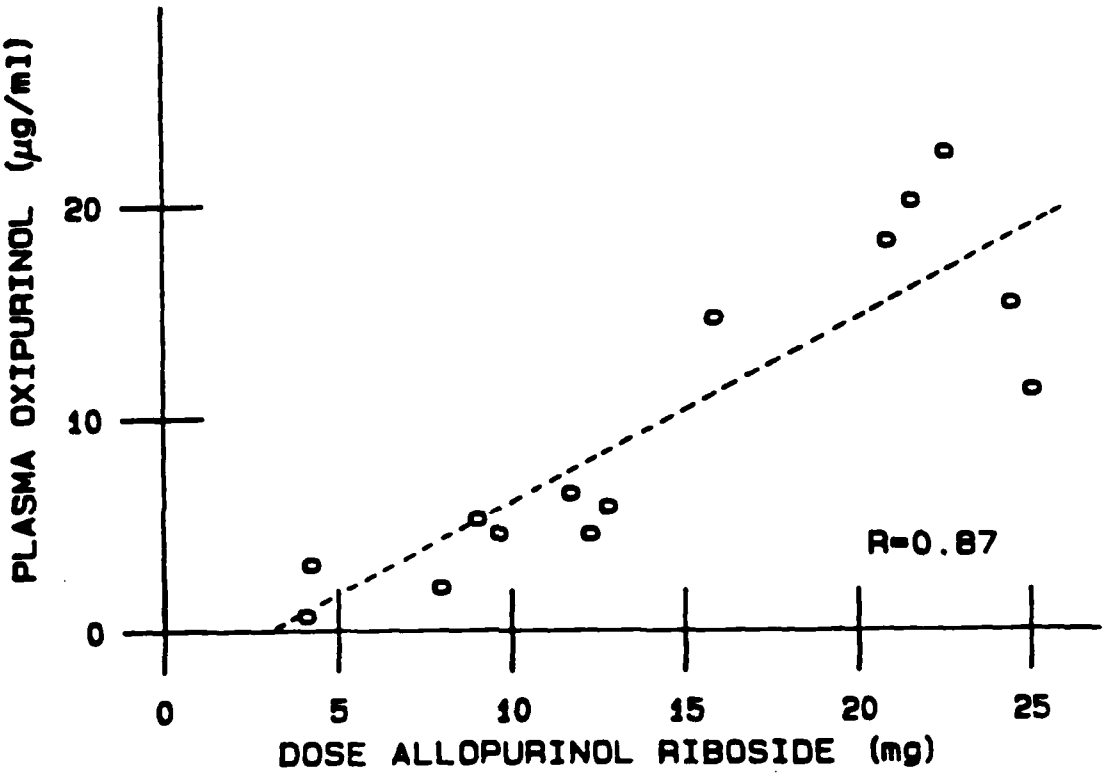


Figure 5. Steady state plasma concentrations of oxipurinol versus dose of allopurinol riboside. Plasma concentrations of oxipurinol on days 2 and 3 were averaged. There is a convincing dose-dependence, and may be a threshold.

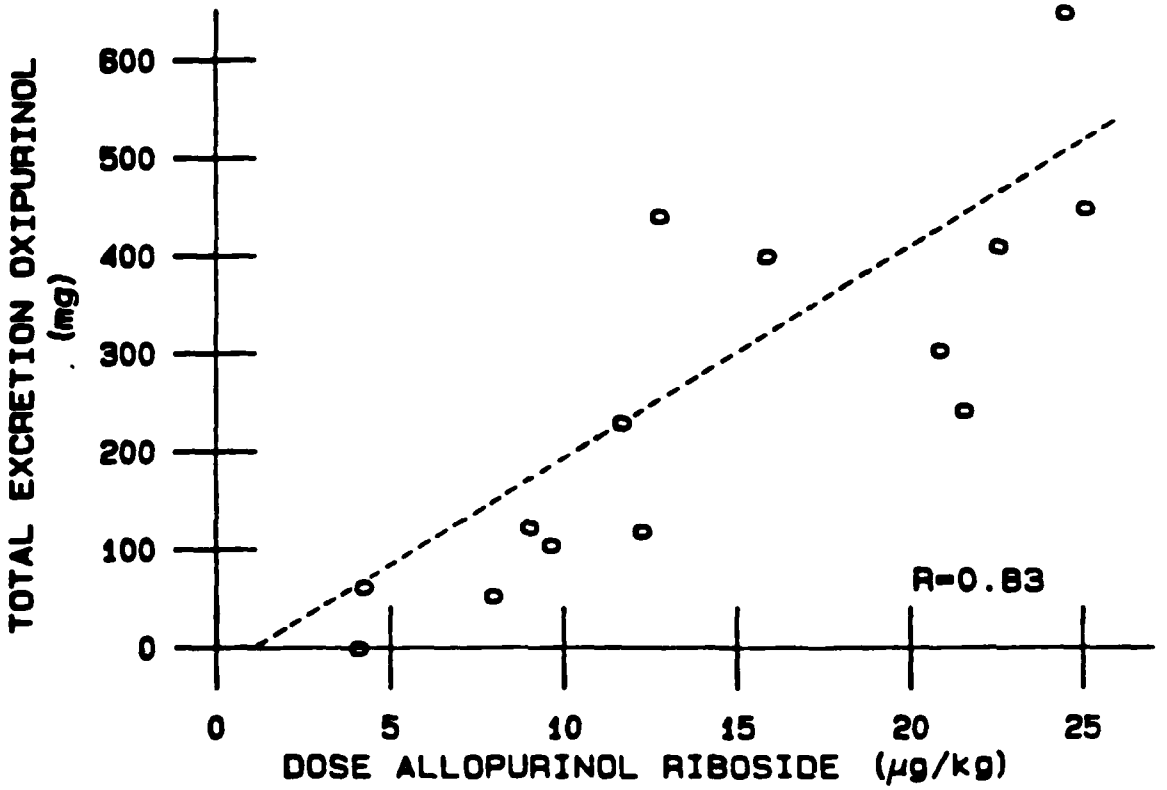


Figure 6. Urinary excretion of oxipurinol versus dose. Urine was collected for 24 hours after the final dose of allopurinol riboside. Dose-dependence is evident.

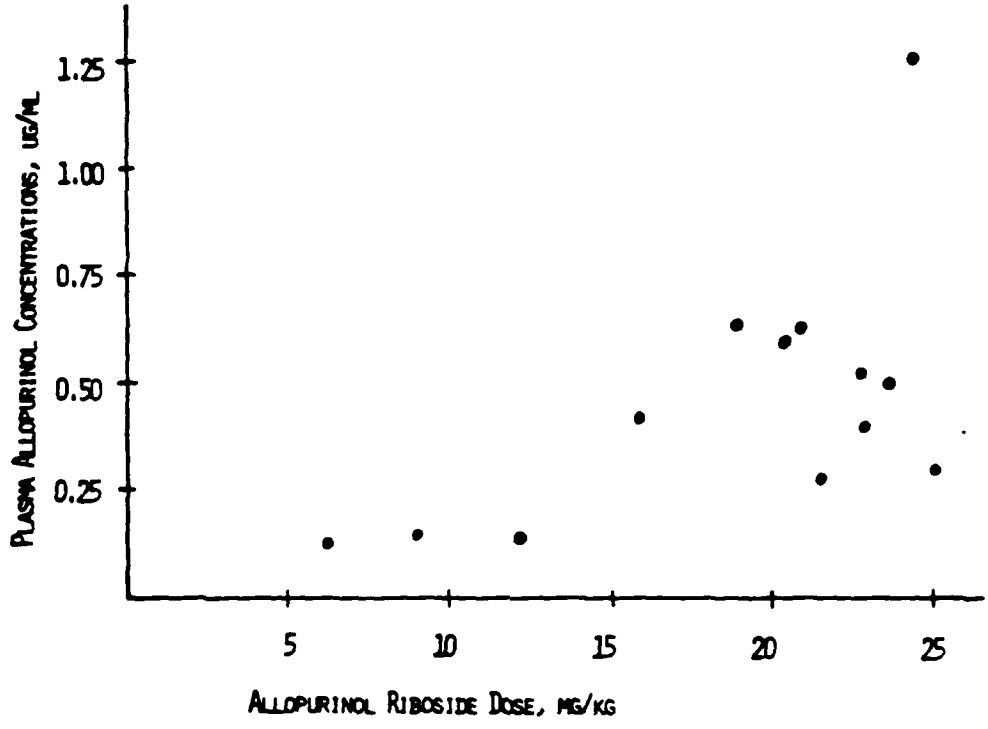


Figure 7. Steady state plasma concentrations of allopurinol. All measurable concentrations of allopurinol, up to and including those from three hours after the final dose of allopurinol riboside were averaged for each volunteer.

Appendix A.
**Procedural Timetable**

| | Prestudy | | Drug Administration | |
|---|---|---|---|---|
| | Day 1 in Clinical Research Unit | Prior to first dose of each day of therapy (Days 2,3,4,5 in Clinical Research Unit) | Day 2 in the Clinical Research Unit at 20 min, 45 min, 75 min, 2, 3, 5, 15 hrs. after the first dose | Days 3 and 4 in the Clinical Research Unit 5 and 15 hrs. after the first dose |
| Physical Examination | X | | | |
| Informed Consent | X | | | |
| Clinical Evaluation* | X | X | X | X |
| Blood Pressure | X | X† | X† | X† |
| Temperature | X | X† | X† | X† |
| Respirations, pulse | X | X† | X† | X† |
| Serum Sample for Pharmacokinetic Study | | X | X | X |
| Urine Sample for Pharmacokinetic Study** | | | X** | |
| Clinical Chemistries: | | | | |
| Serum sodium | X | X | | |
| potassium | X | X | | |
| chloride | X | X | | |
| bicarbonate | X | X | | |
| alkaline phosphatase | X | X | | |
| SGOT | X | X | | |
| SGPT | X | X | | |
| BUN | X | X | | |
| Creatinine | X | X | | |
| Uric acid | X | X | | |
| Total bilirubin | X | X | | |
| Hematology: | | | | |
| Hemoglobin | X | X | | |
| Hematocrit | X | X | | |
| WBC with differential | X | X | | |
| Platelet count | X | X | | |
| Prothrombin time | X | X | | |
| Urinalysis: | | | | |
| Casts | X | X | | |
| Crystals | X | X | | |
| RBC, WBC | X | X | | |
| Glucose | X | X | | |
| Protein | X | X | | |

*Includes complete history and physical examination prior to entry in study, and electrocardiogram only at day 1 (prestudy) in Clinical Research Unit. Physical examination will include an evaluation of the neurology and mental status of the volunteer prior to study entry; in addition, the sleeping and bowel habits will be noted.

**A single urine specimen will be obtained prior to the first dose on day 2 in the Clinical Research Unit. A 24-hour timed, pooled urine collection will start with the first dose on day 2 in the CRU. On day 5 in the CRU, urine will be collected and pooled from 0-2, 2-5, 5-8 and 8-24 hours after drug administration.

†Blood pressure, temperature, respirations and pulse data will be obtained on days 1 through 4 of therapy just prior to drug administration at 7 a.m., 12 p.m., 5 p.m., 10 p.m.

Appendix A.
## Procedural Timetable, Continued

| | Day 5 in Clinical Research Unit at 20 min, 45 min, 75 min, 2 hr, 3 hr, 5 hr, 8 hr, 12 hr, 15 hr, 24 hr After Dosing | Follow-up Period 1 week+ | 2 weeks+ |
|---|---|---|---|
| Physical Examination | | | |
| Informed Consent | | | |
| Clinical Evaluation | X | X | X |
| Blood Pressure | X† | X | X |
| Temperature | X† | X | X |
| Respirations, pulse | X† | X | X |
| Serum Sample for Pharmacokinetic Study | X | | |
| Urine Sample for Pharmacokinetic Study** | X** | | |
| Clinical Chemistries: | | | |
| Serum sodium | | X | X |
| potassium | | X | X |
| chloride | | X | X |
| bicarbonate | | X | X |
| alkaline phosphatase | | X | X |
| SGOT | | X | X |
| SGPT | | X | X |
| BUN | | X | X |
| Creatinine | | X | X |
| Uric acid | | X | X |
| Total bilirubin | | X | X |
| Hematology: | | | |
| Hemoglobin | | X | X |
| Hematocrit | | X | X |
| WBC with differential | | X | X |
| Platelet count | | X | X |
| Prothrombin time | | X | X |
| Urinalysis: | | | |
| Casts | | X | X |
| Crystals | | X | X |
| RBC, WBC | | X | X |
| Glucose | | X | X |
| Protein | | X | X |

**A single urine specimen will be obtained prior to the first dose on day 2 in the Clinical Research Unit. A 24-hour timed, pooled urine collection will start with the first dose on day 2 in the CRU. On day 5 in the CRU, urine will be collected and pooled from 0-2, 2-5, 5-8 and 8-24 hours after drug administration.

+Evaluation plus or minus one day of scheduled visit allowed.

†Blood pressure, temperature, respirations and pulse data will be obtained prior to drug administration at 7 a.m. and at 12 p.m., 5 p.m. and 10 p.m.

APPENDIX B

Publications Supported by This Contract

T. A. Shapiro, J. B. Were, K. Danso, L. E. Rocco, D. J. Nelson, and P. S. Lietman. "Pharmacokinetics and Metabolism of Allopurinol Riboside in Healthy Volunteers". ABSTRACT American Society for Clinical Pharmacology and Therapeutics, Eighty-fifth Annual Meeting. Clin. Pharmacol. Ther. 35: 274, 1984.

## APPENDIX C

Personnel Receiving Contract Support:

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Paul S. Lietman, Investigator
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